Expression of Pemphigoid Antigen by SV40-Transformed Human Keratinocytes

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The expression and properties of pemphigoid antigen of SV40-transformed human keratinocytes were studied. By indirect immunofluorescence, SV40-transformed keratinocytes in passage 80–85 expressed the pemphigoid antigen as coarsely granular perinuclear fluorescence. To characterize this antigen, NP40 extracts of cells labeled with [^{14}C]amino acids were immunoprecipitated using sera of 8 patients: bullous pemphigoid (6 patients), chronic localized pemphigoid (1 patient), and drug-induced lichen planus pemphigoides (1 patient). These immunoprecipitates were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis and then fluorographed. All 8 sera precipitated a protein of M_r 240K, while normal human sera did not precipitate this protein. These results indicate that SV40-transformed human keratinocytes synthesize pemphigoid antigen, and that autoantibodies in the sera of pemphigoid patients with different clinical features identify the same antigen of M_r 240K in these cells. *J Invest Dermatol 86:399–401, 1986*

Pemphigoid antigen is a normal component of the basement membrane zone of epidermis and other squamous epithelia [1], and is known to be synthesized by epidermal basal cells and cultured keratinocytes [2,3]. This antigen is considered to be involved in the pathogenesis of bullous pemphigoid (BP) [4] and the terminal differentiation of epidermal keratinocytes [5]. The recent work of Stanley et al [2] showing that the antigen recognized by several BP sera all had the same molecular weight, suggest that there is only a single antigen. This antigen can be identified immunologically by the circulating autoantibodies of BP patients. While there are some atypical subepidermal bullous diseases that show immunologic features of BP [6,7], the exact nature of their antigens has not been made clear.

It has been shown that human epidermal keratinocytes, following infection by the oncogenic virus SV40, lose the ability to differentiate in an orderly fashion in vitro, and the keratinocytes continue to exhibit stem cell-like characteristics [8].

We have recently had the opportunity to study a case of chronic localized pemphigoid and a rare case of drug-induced lichen planus pemphigoides. In the present study we tried to identify the antigens in SV40-transformed keratinocytes using the sera of patients with BP and these unusual clinical variants.

MATERIALS AND METHODS

Cell Culture Human epidermal keratinocytes derived from neonatal foreskins were grown by the method of Rheinwald and Green [9]. The cultures were infected with SV40 as described in detail previously [10]. Following infection, the cultures were maintained in Dulbecco's modified minimal essential medium supplemented with 10% fetal calf serum and routinely passaged at split ratios of 1:4. The results reported here were based on experiments on a representative cell line (line 130).

Sera Sera were collected from 8 patients: BP (6 patients), chronic localized pemphigoid (1 patient), and drug-induced lichen planus pemphigoides (1 patient). In these cases the diagnoses were made clinically and histologically. Circulating IgG antibasement membrane zone antibodies were detected at high titers in all cases (>320), using normal human skin sections as substrate. In all of these 6 BP patients, linear immunoglobulins and complement deposition could be detected along the basement membrane zone of the lesions by direct immunofluorescent staining. A case with chronic localized pemphigoid showed recurrent bullae formation just on the sural aspect of her right leg. Lesions remained localized for 5 years and resolved spontaneously in September 1984. She has remained free of lesions since then. Results of direct immunofluorescence performed on lesional bullous as well as erythematous skin sections showed no deposition of immunoglobulins and components of complement. The details of a rare case with drug-induced lichen planus pemphigoides have been previously reported [11].

All of these sera were stored at −80°C until use.

Immunofluorescence SV40-infected keratinocytes at the 80th passage were grown on glass coverslips. These cells were fixed with ice-cold methanol and acetone, and indirect immunofluorescence was performed as previously described [2].

Radioactive Labeling and Immunoprecipitation To demonstrate cellular biosynthesis of pemphigoid antigens, we radiolabeled nearly confluent cultures with a mixture of [^{14}C]amino acids (New England Nuclear, sp act 55 mCi/mmol) at 5 μCi/ml. After a 48-h incubation at 37°C these cells were extracted with 0.5% Nonidet P-40 as previously described [2]. To identify newly synthesized antigens, we used previously described immunoprecipitation techniques [12]. After preabsorption with normal human serum the extracts were incubated with different pemphigoid sera, and antigen–antibody complexes were precipitated with

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Abbreviations:
BP: bullous pemphigoid
SDS-PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis

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protein A bearing staphylococci (Pansorbin, Calbiochem Behring, La Jolla, California). The immunoprecipitated pemphigoid antigens were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a 5% gel for resolving the antigens. Following electrophoresis, gels were fluorographed.

RESULTS

Indirect Immunofluorescence The keratinocyte cultures used in these experiments were between the 80th and 85th serial passage postinfectiton. In these cultures, SV40-transformed keratinocytes did not undergo orderly stratification and synthesis of mature squames which characterize the nontransformed cells [13,14].

By indirect immunofluorescent staining, SV40-transformed keratinocytes displayed pemphigoid antigens in a perinuclear granular pattern, which probably represents intracellular staining (Fig 1). These antigens were detected in almost 100% of the cells. All of the 8 different sera recognized the antigens in these cells with essentially the same staining pattern, which is similar to those described for other cell types (i.e., Pam cells, primary epidermal keratinocytes grown in a low Ca^{++} medium) [2,5]. Normal human sera demonstrated no specific staining on these cells. These findings demonstrate that pemphigoid antigen is present and probably synthesized in SV40-transformed human keratinocytes.

Immunoprecipitation To demonstrate directly that SV40-transformed keratinocytes synthesize pemphigoid antigen, we radiolabeled these cells with [^{14}C]-labeled amino acids, then extracted them with nonionic detergents. Newly synthesized pemphigoid antigen was precipitated from the extracts using 8 different sera or normal human sera as controls. Immunoprecipitated pemphigoid antigens were finally reduced with 5% β-mercaptoethanol and identified by SDS-PAGE and fluorography. All the 8 sera precipitated a protein of M_r 240K from the extract, while normal sera did not precipitate this protein (Fig 2). No other specifically immunoprecipitated bands could be seen on these gels. Thus it was found that SV40-transformed human keratinocytes synthesize pemphigoid antigen, and that autoantibodies in the sera of patients with BP, chronic localized pemphigoid, and drug-induced lichen planus pemphigoides identify the same antigen of M_r 240K.

DISCUSSION

The findings of this study demonstrate that SV40-transformed human keratinocytes synthesize pemphigoid antigen. This property which expresses the nature of the basal cell is consistent with that of the Pam cells [2] or Kirsten and Harvey sarcoma virus-infected keratinocytes [15]. It has been shown that after SV40 infection human keratinocytes express progressive enhancement of growth characteristics and inhibition of normal differentiation process in vitro. Among these changes are growth properties generally associated with transformation in vitro [8,10] as well as a number of transformed properties associated specifically with expression of the epidermal phenotype, e.g., loss of the ability to synthesize involucrin, form cornified cell envelopes, and stratify [8,10,13]. Also, it has been shown that, when inoculated s.c. into nude mice, SV40-transformed keratinocytes form cysts which exhibit histologic features closely resembling those seen in undifferentiated squamous cell carcinomas [16]. However, other properties appear to be unique to transformation by the virus. For example, the viral transformants express a keratin pattern corresponding to that in simple epithelia whereas cell lines derived from squamous cell carcinomas stably maintain a keratin pattern characteristic of stratified epithelium [17,18]. The depletion of specific antigens within the epidermis such as pemphigus and pemphigoid antigens has been considered as an important factor in neoplastic behavior [19]. There have been several reports that the tumor cells from invasive squamous cell carcinomas and basal cell carcinomas lose the ability to synthesize these antigens [19,20]. Thus the continued synthesis of pemphigoid antigen by the viral transformants may also be of interest as a feature of the epidermal phenotype whose altered expression may correlate with aspects of the transformation process in vivo which are not induced during transformation by SV40.

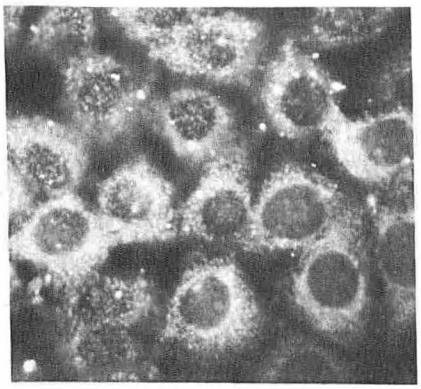

Figure 1. Indirect immunofluorescence of pemphigoid antigen in SV40-transformed human keratinocytes (at passage 82). × 220.

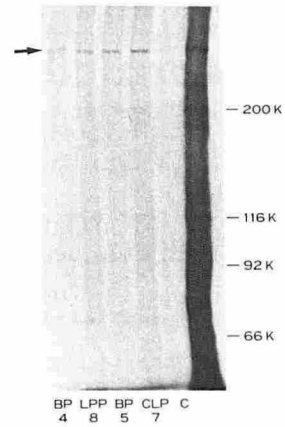

Figure 2. Immunoprecipitation of pemphigoid antigen from radiolabeled extracts of SV40-transformed keratinocytes. *Right lane* indicates the electrophoretic pattern of total extracts with 0.5% Nonidet P-40. Sera of patients with bullous pemphigoid (*BP*), chronic localized pemphigoid (*CLP*), and lichen planus pemphigoides (*LPP*) identify a band of approximately 240K (*arrow*). Normal control serum (*C*) does not precipitate this protein.

The observations that different patients with BP have antibodies against the same protein are consistent with those reported previously. Our present work also demonstrates that the antigen recognized by the sera from the patients with chronic localized pemphigoid and drug-induced lichen planus pemphigoides is the same in molecular weight as that of BP. The case with localized pemphigoid described herein had a circulating antibasement membrane zone antibody at a high titer. Although the question of why the blistering process develops only in a localized area cannot be answered at present, the antigenic difference does not exist between localized and generalized BP. According to the report of Person et al [6], among 9 cases with localized pemphigoid, generalized BP eventually developed in 3 of them. Considering the findings of their follow-up study together, our present results emphasize that localized pemphigoid is indeed a clinical variant of BP. Although the case with lichen planus pemphigoides was clinically atypical, its immunologic feature was typical of BP. BP antigen synthesized in these transformed epidermal keratinocytes was also supposed to be involved in the pathogenesis of this rare case. These findings suggest that despite its unusual clinical manifestations, lichen planus pemphigoides seems to be closely related to BP.

REFERENCES

1. Beutner EH, Jordon RE, Chorzelski TP: The immunopathology of pemphigus and bullous pemphigoid. J Invest Dermatol 51:63–80, 1968
2. Stanley JR, Hawley-Nelson P, Yuspa SH, Shevach EM, Katz SI: Characterization of bullous pemphigoid antigen: a unique basement membrane protein of stratified squamous epithelia. Cell 24:897–903, 1981
3. Stanley JR, Hawley-Nelson P, Yaar M, Martin GR, Katz SI: Laminin and bullous pemphigoid antigen are distinct basement membrane proteins synthesized by epidermal cells. J Invest Dermatol 78:456–459, 1982
4. Sams WM, Gammon WR: Mechanism of lesion production in pemphigus and pemphigoid. J Am Acad Dermatol 6:431–449, 1982
5. Stanley JR, Yuspa SH: Specific epidermal protein markers are modulated during calcium-induced terminal differentiation. J Cell Biol 96:1809–1814, 1983
6. Person JR, Rogers RS III, Perry HO: Localized pemphigoid. Br J Dermatol 95:531–533, 1976
7. Provost TT, Maize JC, Ahmed AR, Strauss JS, Dobson RL: Unusual subepidermal bullous diseases with immunologic features of bullous pemphigoid. Arch Dermatol 115:156–160, 1979
8. Steinberg ML, Defendi V: Transformation and immortalization of human keratinocytes by SV40. J Invest Dermatol 81:131–136, 1983
9. Rheinwald J, Green H: Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. Cell 6:331–334, 1975
10. Steinberg ML, Defendi V: Altered pattern of growth and differentiation in human keratinocytes infected by simian virus 40. Proc Natl Acad Sci USA 76:801–805, 1979
11. Miyagawa S, Ohi H, Okuchi T, Shirai T, Sakamoto K: Lichen planus pemphigoides-like lesions induced by cinnarizine. Br J Dermatol 112:607–613, 1985
12. Kessler SW: Cell membrane antigen isolation with the staphylococcal protein A-antibody absorbent. J Immunol 117:1482–1490, 1976
13. Okada N, Steinberg ML, Defendi V: Re-expression of differentiated properties in SV40-infected human epidermal keratinocytes induced by 5-Azacytidine. Exp Cell Res 153:198–207, 1984
14. Mufson RA, Steinberg ML, Defendi V: Effects of 12-*O*-Tetradecanoyl-phorbol-13-acetate on the differentiation of simian virus 40-infected human keratinocytes. Cancer Res 42:4600–4605, 1982
15. Yuspa SH, Kilkenny AE, Stanley JR, Lichti U: Keratinocytes blocked in phorbol ester-responsive early stage of terminal differentiation by sarcoma viruses. Nature 314:459–462, 1985
16. Steinberg ML, Morris A, Goodman S: Oncogenic properties of human keratinocytes transformed by SV40, Processes in Cutaneous Epidermal Differentiation. Edited by IA Bernstein, T Hirone. Treager Scientific Publishing, in press
17. Hronis TS, Steinberg ML, Defendi V, Sun TT: Simple epithelial nature of some simian virus-40-transformed human epidermal keratinocytes. Cancer Res 44:5797–5804, 1984
18. Steinberg ML, Defendi V: Altered patterns of keratin synthesis in human epidermal keratinocytes transformed by SV40. J Cell Physiol 123:117–125, 1985
19. Toska A, Varelzidis A, Nicolis G, Hadzis J, Stratigos J, Capetanakis J: Antigenic alteration in tumors of epidermal origin. Cancer 45:2284–2290, 1980
20. Stanley JR, Beckwith JB, Fuller RP, Katz SI: A specific antigenic defect of the basement membrane is found in basal cell carcinoma but not in other epidermal tumors. Cancer 50:1486–1490, 1982